Issued April 15, 1913.

U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF ANIMAL INDUSTRY.—BULLETIN 167.

A. D. MELVIN, CHIEF OF BUREAU.

THE ACTION OF ARSENICAL DIPS IN PROTECTING CATTLE FROM INFESTATION WITH TICKS.

BY

H. W. GRAYBILL, D. V. M.,
Assistant Zoologist, Zoological Division.

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LETTER OF TRANSMITTAL.

United States Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., January 23, 1913.

Sir: I have the honor to transmit for publication as a bulletin of this bureau the accompanying manuscript entitled "The Action of Arsenical Dips in Protecting Cattle from Infestation with Ticks," by Dr. H. W. Graybill, of the Zoological Division of this bureau.

The work herein described was undertaken mainly for the purpose of determining to what extent and how long an arsenical dip protects cattle from infestation with ticks after dipping. Three experiments were conducted with cattle, by which it was found that infestation was prevented for two days, and that there was no protection when the exposure to infestation occurred five days or longer after dipping. Further experiments to ascertain if the protection extends beyond two days are in progress and will be reported later.

The present work includes a series of experiments made with cattle ticks to determine the manner in which substances used in dips (oils and arsenic) act upon them. It is shown that ticks are destroyed by dips either by suffocation or by poisoning, or by both.

Respectfully,

A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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THE ACTION OF ARSENICAL DIPS IN PROTECTING CATTLE FROM INFESTATION WITH TICKS.

SUMMARY.

In this bulletin the factors entering into the efficacy of dips used against ticks are discussed. Dips act both in a direct destructive way and in a protective manner preventing infestation. The protective action of a dip may be in the nature of a destructive or of a repellent action. The influence of dips on oviposition and the viability of the eggs is a factor in efficacy.

The ingredients of homemade arsenical dips and the known or probable functions of each ingredient are discussed.

It is suggested herein that any protective action that the usual arsenical dips might have would be expected to be due to a toxic rather than to a repellent action. Watkins-Pitchford has shown that cattle dipped in arsenic are poisonous to ticks.

Ticks are destroyed by dips either by suffocation or by poisoning, or by both means. Tests were conducted showing that ticks are suffocated by the closing of the respiratory openings (spiracles). It was found that practically all engorged females that had their spiracles closed with Canada balsam died. In other tests of the same sort, in which oils were used, Beaumont oil proved much less effective than Canada balsam, and cottonseed oil was practically without effect.

Smearing the scutum and mouth parts of engorged females with oils and viscous substances had no influence on the mortality, or oviposition, or on the per cent of eggs hatching.

In tests in which engorged females were dipped in Beaumont oil and in cottonseed oil the former proved very much more effective than the latter, and this is due in all probability to a toxic action. Beaumont oil had a marked influence on oviposition, on the number of eggs deposited, and on the viability of the eggs, whereas cottonseed oil had no effect.

The possible avenues for the entrance of arsenic into the bodies of ticks are enumerated, and the porose areas are pointed out as possible vulnerable points in the armor of the tick.

Three cattle-dipping experiments were conducted with an arsenical dip containing 8 pounds of arsenic trioxid to 500 gallons of water, in order to test its protective action against tick infestation. Seed ticks were placed on the cattle at varying periods after they were dipped. In the first experiment the ticks were placed on the cattle at periods ranging from a few hours to four weeks, in the second from a few hours to two days, and in the third at five days after dipping. It was found that the dip rendered no protection when the exposure to infestation was five days or longer after dipping. The limit of protection ascertained in the experiments was two days. No tests were made covering the intervening period between two and five days.

Arsenical poisoning which occurred among the animals in one experiment was apparently caused by undissolved arsenic in the dip. It would therefore seem that undissolved arsenic in a dip is highly dangerous.

It is shown conclusively that the protective action of arsenic is dependent on its toxic action, and not on a repellent action.

As a result of incidental observations made on engorged female ticks from animals suffering from Texas fever it was found that the mortality of such ticks may be very high, as much as 95 per cent. The cause for this is not known. It may be nutritional in character, due to the changed or impoverished condition of the blood absorbed, or it may be due to the parasitism of *Piroplasma bigeminum*, the microorganism which is the direct cause of Texas fever.

Observations made for the purpose of determining whether there was any relationship between the degree of infestation and the time elapsing between the last dipping and the infestation, and also on the mortality of engorged females from dipped animals infested subsequently to dipping, as compared with that of ticks from undipped animals, were inconclusive. Oviposition and viability of the eggs of these ticks appeared to be unaffected.

INTRODUCTION.

The efficacy of a dip used against such a pest as the cattle tick may be considered under two heads, namely, its direct destructive action, and its protective action in preventing reinfestation. (Protective action may be the result either of a destructive or of a repellent action, or of both.) These properties are no doubt combined to a certain degree in all dips that have proved effective against ticks. The relative importance that they assume, from a practical standpoint, depends on the particular purpose for which dipping is done. If it is desired to render cattle free of ticks, and they are not to be subjected to the dangers of reinfestation, the protective value of the dip is of minor importance. On the other hand, if the purpose is to eradicate the ticks from a given area, and the animals are turned back on infested fields subsequent to each treatment, the protective value of a dip assumes considerable importance. In the former case the protective properties would be of value only in case the animals should by accident be subjected to infestation subsequent to dipping, whereas in the latter case they would play an important part in bringing about extermination. It is conceivable, though we have no such dips at present, that a dip might stand low in its direct destructive action and still prove highly effective in eradication work because of its protective (repellent) properties.

In addition to the destructive and protective qualities of a dip another factor must be considered, and that is its influence on oviposition and on the viability of the eggs deposited. It has been pointed out in Bulletin 144 of the Bureau of Animal Industry that arsenic diminishes both the number of eggs deposited by females that survive and to a marked degree the viability of the eggs. Certain oils also affect the number of eggs deposited and the viability of the eggs. Various oils are known to be more or less effective against ticks, and they all no doubt possess a certain amount of protective value, dependent on their odor, their disagreeable nature, or the destructive

action they exert when seed ticks come in contact with them. Other things being equal, the less volatile the oil the longer the protective action lasts.

The arsenical dips which have shown a high degree of efficacy against ticks were commonly supposed to have little or no protective value until Watkins-Pitchford[1] in 1910 showed by experiments that animals dipped in arsenic are poisonous to ticks. The same author[2] in other experiments found that 16.8 per cent of the adult ticks that became attached to cattle shortly after dipping in arsenic (4 pounds of arsenite of soda (80 per cent arsenic), 3 pounds of soft soap, and 1 gallon of paraffin to make 400 gallons of dip) were dead at the end of three days, and that in the case of horses 22.5 per cent of the ticks were dead. In one experiment in which horses had been frequently dipped the tick mortality was 36.8 per cent among ticks that attached during the course of three days.

Arsenical dips containing sodium metarsenite in alkaline solution and an admixture of pine tar could not be expected to exert any repellent action because of their physical nature or their odor, except in so far as the tar is concerned, the action of which, however, must be very slight. Any protective value that such dips have, therefore, must necessarily depend largely on the toxicity of the arsenic present on or in the skin of dipped animals. Watkins-Pitchford[2] found that the arsenical dip above mentioned retarded infestation with larvæ and nymphs of the brown tick. The time between treatment and infestation is not stated, but it was presumably only a few hours. The author attributed the protective action to the paraffin component of the dip. It was found that dipping did not hinder infestation with the adult of the brown tick, but that the number on cattle subjected to infestation rapidly decreased as a result of the poisonous effect of the arsenic present in the skin of the animals.

In the work of tick suppression and tick eradication in this and other countries arsenical dips have proved satisfactory when used at regular intervals. The strength of the dip and the interval between treatments have been determined with a view of obtaining the best results with as little injury to the cattle as possible. The interval between dippings has rested largely on an empirical basis, except in the case of treatment directed against certain species of ticks of economic importance in South Africa that drop from their host previous to each molt, in which case the period has been based in some instances on the minimum time the tick has been found to remain attached to the host. Little is known as to how much the ar-

[1] Natal Agricultural Journal, Pietermaritzburg, vol. 15, no. 3, Sept., 1910, pp. 312–328.

[2] Agricultural Journal, Union of South Africa, Pretoria, vol. 2, no. 1, July, 1911, pp. 33–79. Rhodesia Agricultural Journal, Salisbury, vol. 10, no. 3, Feb., 1912, pp. 372–400.

senical dip plays in a protective way in bringing about the results that have been attained. It was with a view of determining to what extent and for how long arsenic protects against infestation that the experiments detailed in this paper were taken up.

COMPOSITION OF ARSENICAL DIPS.

Arsenic seems to have almost a specific action on ticks. The arsenical dips in use against ticks as a rule have their arsenic present in the form of sodium or potassium metarsenite, the former of which, however, in the presence of bacterial growth, as shown by Fuller,[1] gradually becomes oxidized to the arsenate. In the home-made dips used in this country the only other ingredients are an excess of sodium carbonate and pine tar. In formulas that were formerly tried in this country, and some now in use in other countries, there are other ingredients, such as soap and emulsified paraffin (kerosene).

Watkins-Pitchford[2] has found that paraffin emulsified with soap in the dip ameliorates its irritative action and that the addition of emulsified paraffin and glycerin subdues this action still more. It is not likely that the soap, pine tar, or paraffin exert any important destructive action on ticks, especially in view of the fact that they constitute less than 1 per cent of the dip. Watkins-Pitchford[3] found that the mortality of ticks in the case of two arsenical dips was raised 1.8 and 5.7 per cent by the addition of soap and paraffin. His observations, however, were based on too small a number of ticks to be considered conclusive. All three of the ingredients mentioned may serve a certain function in causing the arsenic to adhere more tenaciously to the skin and hair, and the tar and paraffin may exert a slight amount of repellent action because of their odor and physical properties. They also serve the purpose of giving the dip a distinctive character. The soap serves a function in the emulsification of the tar or kerosene, depending on which is used, and probably exerts a certain amount of cleansing effect on the skin.

The excess of sodium carbonate in the two formulas in use in this country, in which 8 and 10 pounds of white arsenic are used to 24 and 25 pounds, respectively, of sodium carbonate, is considerable. Whereas only 1.45 parts by weight of sodium carbonate to 1 part of arsenic are necessary in the formation of sodium metarsenite, in the above formulas 2½ to 3 parts are used to each part of arsenic. However, this excess of sodium carbonate serves a number of important purposes. It facilitates the solution of the arsenic, aids in the emul-

[1] Circular 182, Bureau Animal Industry, U. S. Department of Agriculture. Washington, 1911.

[2] Natal Agricultural Journal, Pietermaritzburg, vol. 12, no. 4, Apr. 30. 1909.

[3] Agricultural Journal, Union of South Africa, Pretoria, vol. 2, no. 1, July, 1911, pp. 33–79. Rhodesia Agricultural Journal, Salisbury, vol. 10, no. 3, Feb., 1912, pp. 372–400.

sification of the tar, cleanses the skin of the cattle, and possibly softens the cuticle of the ticks and thus facilitates the entrance of arsenic.

THE MANNER IN WHICH DIPS ACT ON TICKS.

When ticks are destroyed by a dip they are killed by suffocation, by being poisoned, or by a combination of both causes. In the case of the use of such substances as oils it is probable that death is due both to suffocation and to a toxic action.

THE ACTION OF OILS AND OTHER SUBSTANCES ON TICKS.

SMEARING THE SPIRACLES.

That ticks may be suffocated the writer has shown by closing the spiracles of engorged females of the cattle tick with Canada balsam. On May 19, 1911, 10 engorged females that had already begun to oviposit had their spiracles closed with a drop of balsam. On the following day the ticks were normal in color but were inactive, their legs being stretched out straight. On the third day all the ticks had turned dark and were dead. No eggs were deposited. In the control lot of 10 ticks 4 died. Many eggs were deposited, 40 per cent of which hatched.

In order to determine whether the xylol in the balsam may not have played a part in the destruction of the ticks a second test was made on May 26 in which the spiracles of 10 engorged females that had begun to oviposit were touched with xylol. One tick turned dark. All oviposited, and a normal number of eggs were deposited, about 30 per cent of which hatched.

On September 27, 1912, another lot of 20 engorged females just collected had their spiracles touched with Canada balsam. On September 30 all of them were dead. In two control lots of 20 engorged females each, collected on the same date, all the ticks remained normal and oviposited; 92 and 60 per cent of the eggs hatched.

On September 29, 1912, a third lot of 20 engorged females, collected on September 27, 1912, had their spiracles touched with Canada balsam. Seventeen of the ticks were killed, the remainder deposited normal eggs. Eighty-seven per cent of the eggs hatched. In two control lots of 20 ticks each, collected on the same date as the above lot, all oviposited and a usual number of normal eggs were deposited; 92 and 80 per cent of the eggs hatched.

From the above tests it may be definitely concluded that a complete closing of the spiracles of engorged females will lead to their destruction. In the case of the three ticks in the last lot that survived it seems safe to assume, in view of the number of positive tests, that the spiracles were not completely closed.

Some additional tests were made along the above line with other oils. On May 13, 1911, eight engorged females that had already begun to oviposit had their spiracles smeared with Beaumont crude petroleum. All but one continued oviposition. Three of the ticks died. Many eggs were deposited, 25 per cent of which hatched. In a control lot of seven ticks all continued oviposition. Four ticks died. A fair number of eggs were deposited, about 30 per cent of which hatched.

September 27, 1912, 20 engorged females collected on that date had their spiracles touched with crude Beaumont oil. One (5 per cent) of the ticks died. It had apparently been injured about the head when removed from the host. Three ticks remained plump. Nineteen ticks oviposited, and a normal number of eggs were produced. Seven per cent of the eggs hatched.

September 27, 1912, 20 engorged females just collected had their spiracles touched with refined cottonseed oil. None died. All oviposited, and a normal number of eggs were deposited. Ninety-two per cent of the eggs hatched.

Two control lots of 20 ticks each, collected on the same date as the above, remained normal. All the ticks oviposited, and a normal number of eggs were produced; 92 and 60 per cent of the eggs hatched.

On September 27, 1912, 20 engorged females just collected had their spiracles touched with Beaumont oil. One (5 per cent) failed to oviposit. Three (15 per cent) died. A normal number of eggs were deposited. Twenty per cent of the eggs hatched.

On September 28, 1912, 20 ticks collected on September 27 had their spiracles touched with refined cottonseed oil. None of the ticks died. All the ticks oviposited, and a normal number of eggs were deposited. Ninety per cent of the eggs hatched.

In two control lots of 20 ticks each, collected on the same date as the above, none died, and all oviposited, a normal number of eggs being produced; 80 and 92 per cent of the eggs hatched.

On May 26, 1911, 10 engorged females that had begun to oviposit had their spiracles touched with pine tar. All oviposited, and a normal number of eggs were deposited, 15 per cent of which hatched. In the control lot of 10 ticks, all but one oviposited, and five died without completing oviposition. Many eggs were deposited, 10 per cent of which hatched.

Summary of tests in which spiracles of engorged females were smeared with oil.

Date of experiment.	Mortality.	Ticks ovipositing.	Number of eggs deposited.	Eggs hatched.	Substance used.
	Per cent.	*Per cent.*		*Per cent.*	
May 13, 1911	38	88	Many	25	Beaumont oil.
Do	57	100	Fair	30	(Control.)
Sept. 27, 1912	5	95	Normal	7	Beaumont oil.
Do	0	100	do	92	Cottonseed oil.
Do	0	100	do	92	(Control.)
Do	0	100	do	60	Do.
Do	15	95	do	20	Beaumont oil.
Sept. 28, 1912	0	100	do	90	Cottonseed oil.
Sept. 27, 1912	0	100	do	80	(Control.)
Do	0	100	do	92	Do.
May 26, 1911		100	do	15	Pine tar.
Do	50	90	Many	10	(Control.)

It is noted from the above table that the mortality of the different lots of ticks treated with Beaumont oil compared with that of the corresponding controls is as follows: 38 per cent, control 57 per cent; 5 per cent, controls 0 per cent; 15 per cent, controls 0 per cent. This indicates a detrimental effect of the Beaumont oil, but is in marked contrast with the results obtained when the spiracles were closed with Canada balsam, in which case the mortality was practically 100 per cent. The mortality in the case of cottonseed oil was 0. Beaumont oil, therefore, appears to be much more effective than cottonseed oil in closing the spiracles, although it should be borne in mind that there may be a toxic effect that entered into the results. It is evident that Beaumont oil does not close the spiracles as effectually as Canada balsam.

The percentage of ticks in the lots treated with Beaumont oil that oviposited, compared with the percentage in the corresponding controls, is as follows: 88 per cent, control 100 per cent; 95 per cent, controls 100 per cent; 95 per cent, controls 100 per cent. It appears from this that the treatment had a slight effect on oviposition. Cottonseed oil had no effect on the percentage ovipositing. The treatment in no case had any effect on the number of eggs deposited. The percentage of eggs that hatched in the case of the ticks treated with Beaumont oil, compared with the percentage in the corresponding control lots, is as follows: 25 per cent, control 30 per cent; 7 per cent, controls 60 and 92 per cent; and 20 per cent, controls 80 and 92 per cent. From this it appears that the Beaumont oil applied to the spiracles must have been absorbed to some extent and by this means exerted a detrimental influence on the eggs.

The percentage of eggs hatching in the case of ticks treated with cottonseed oil, compared with that of the corresponding controls, is as follows: 92 per cent, controls 60 and 92 per cent; and 90 per cent, controls 80 to 92 per cent. It is therefore noted that cottonseed oil had no influence on the eggs.

SMEARING THE SCUTUM AND MOUTH PARTS.

In order to determine whether the presence of an oil or viscous substance on the scutum and mouth parts would interfere with oviposition and the laying of normal eggs, a number of tests were made. On June 8, 1911, 10 engorged females that had already begun to oviposit were smeared on the scutum and mouth parts with Beaumont oil. Eight of the ticks died. All but one of the ticks deposited some eggs. Only a few eggs were deposited, none of which hatched. In the control lot of 10 ticks, 6 died. All oviposited. A moderate number of eggs were deposited, none of which hatched.

On June 8, 1911, 10 engorged ticks that had begun to oviposit were similarly smeared with Canada balsam. All but one oviposited. Eight ticks died. A moderate number of eggs were deposited, less than 1 per cent of which hatched. In the control lot of ticks, six died. All oviposited. A moderate number of eggs were deposited, none of which hatched.

On June 8, 1911, 10 engorged females that had begun to oviposit were smeared on the scutum and mouth parts with pine tar. All the ticks oviposited. Eight died. A considerable number of eggs were deposited, 10 per cent of which hatched. In the control lot, consisting of 10 ticks, 6 died. All oviposited. A moderate number of eggs were deposited, none of which hatched.

September 27, 1912, 20 engorged females just collected were similarly smeared with crude Beaumont oil. All oviposited. A normal number of eggs were deposited. Fifty per cent of the eggs hatched.

On September 27, 1912, 20 engorged females just collected were smeared on the scutum and mouth parts with refined cottonseed oil. One tick became discolored. Two more ticks became discolored shortly before oviposition was completed. All oviposited. A normal number of eggs were deposited, and 87 per cent of the eggs hatched.

On September 27, 1912, 20 engorged females just collected were similarly smeared with Canada balsam. All oviposited. A usual number of normal eggs were deposited. Seventy-five per cent of the eggs hatched.

In two control lots of 20 ticks each, collected on the same date as the above, none died. All oviposited. A normal number of eggs were deposited; 92 and 60 per cent of the eggs hatched.

On September 27, 1912, the scutum and mouth parts of 20 engorged females just collected were smeared with crude Beaumont oil. One tick (5 per cent) died and one became abnormal in appearance. All but one oviposited. A normal number of eggs were deposited. Eighty-five per cent of the eggs hatched.

On September 28, 1912, the scutum and mouth parts of 20 engorged females collected on the previous day were smeared with refined

cottonseed oil. Five ticks (25 per cent) died. Sixteen ticks oviposited. A normal number of eggs were deposited. Ninety-five per cent of the eggs hatched.

September 29, 1912, the scutum and mouth parts of 20 engorged females collected on September 27 were smeared with Canada balsam. All the ticks lived and oviposited. A usual number of normal eggs were deposited. Fifty per cent of the eggs hatched.

In two control lots of 20 ticks each, collected on the same date as the above, none died. All oviposited and a normal number of eggs were deposited; 92 and 80 per cent of the eggs hatched.

Summary of tests in which the scutum and mouth parts of engorged females were smeared with oils.

Date of test.	Mortality.	Ticks ovipositing.	Number of eggs deposited.	Eggs hatched.	Substance used.
1911.	*Per cent.*	*Per cent.*		*Per cent.*	
June 8	80	90	A few	0	Beaumont oil.
Do	60	100	Moderate	0	(Control.)
Do	80	90	.....do	(1)	Canada balsam.
Do	60	100	.....do	0	(Control.)
Do	80	100	Considerable	10	Pine tar.
Do	60	100	Moderate	0	(Control.)
1912.					
September 27	....	100	Normal	50	Beaumont oil.
Do	[2] 0	100	.....do	87	Cottonseed oil.
Do	0	100	.....do	75	Canada balsam.
Do	0	100	.....do	92	(Control.)
Do	0	100	.....do	60	Do.
Do	[3] 5	95	.....do	85	Beaumont oil.
September 28	25	80	.....do	95	Cottonseed oil.
September 29	0	100	.....do	50	Canada balsam.
September 27	0	100	.....do	92	(Control.)
Do	0	100	.....do	80	Do.

[1] Less than 1 per cent.
[2] Three (15 per cent) became discolored.
[3] One more (5 per cent) became abnormal in appearance.

In the tests in the above table, started on June 8, 1911, the high mortality of the ticks was no doubt due to the fact that they had been sent in from the South and in consequence had been injured to a certain extent. A comparison of the mortality of the treated ticks with that of the corresponding controls indicates that the treatment played no important part, if any, in determining the percentage of mortality. It is likewise apparent that the treatment had practically no influence on the number ovipositing and on the number of eggs deposited, and, so far as can be determined, the same seems to be true with regard to the viability of the eggs.

DIPPING THE TICKS IN OILS.

A number of tests were made in which ticks were dipped in crude Beaumont oil and in refined cottonseed oil.

On September 27, 1912, 20 engorged females just collected were dipped in crude Beaumont oil. Eleven (12?) oviposited. Eight

(40 per cent) died. Only a small number of eggs were deposited, part of which shriveled. Less than 1 per cent of the eggs hatched.

On September 27, 1912, 20 engorged females just collected were dipped in refined cottonseed oil. One tick (5 per cent) died. All oviposited. A normal number of eggs were deposited. Ninety per cent of the eggs hatched.

In two control lots collected on September 27, consisting of 20 ticks each, none died. All oviposited and a normal number of eggs were deposited; 92 and 60 per cent of the eggs hatched.

September 27, 1912, 20 engorged females just collected were dipped in Beaumont crude petroleum. Thirteen ticks (65 per cent) oviposited. Eight ticks (40 per cent) died. Several others became abnormal in appearance. Only a few eggs were deposited, about half of which shriveled. Less than 1 per cent of the eggs hatched.

September 28, 1912, 20 engorged females collected the previous day were dipped in refined cottonseed oil. All but one tick remained normal. A normal number of eggs were deposited, some of which shriveled. Eighty-five per cent of the eggs hatched.

In two control lots of 20 ticks each, collected on September 27, 1912, all oviposited. A normal number of eggs were deposited; 92 and 80 per cent of the eggs hatched.

Summary of tests in which engorged ticks were dipped in oils.

Date of test.	Mortality.	Ticks ovipositing.	Number of eggs deposited.	Eggs hatched.	Oil used.
	Per cent.	*Per cent.*		*Per cent.*	
September 27, 1912	40	55	Small	(1)	Beaumont oil.
Do	5	100	Normal	90	Cottonseed oil.
Do	0	100	do	92	(Control.)
Do	0	100	do	60	Do.
Do	2 40	65	Few	(1)	Beaumont oil.
September 28, 1912	3 0		Normal	85	Cottonseed oil.
September 27, 1912	0	100	do	92	(Control.)
Do	0	100	do	80	Do.

1 Less than 1 per cent.
2 Others became abnormal in appearance.
3 One tick (5 per cent) became abnormal in appearance.

In the foregoing table it is noted that the mortality in the case of ticks dipped in Beaumont oil was 40 per cent in both tests, and in the case of ticks dipped in cottonseed oil it was 5 per cent and 0 per cent. Beaumont oil is therefore shown to have a greater destructive action than cottonseed oil, and this is probably due largely to a toxic action of some kind, since in the tests in which the spiracles were touched with Beaumont oil this oil was shown to be much less effective than in the present tests. Beaumont oil reduced the percentage of females ovipositing and the number of eggs deposited. Cottonseed oil had no influence on oviposition or on the number of eggs deposited. The percentage of eggs hatching in the case of the ticks dipped in Beau-

mont oil was very low—less than 1 per cent—while the percentage hatching in the case of those dipped in cottonseed oil was practically normal. This would seem to be further evidence of the absorption of Beaumont oil and its toxicity, although it should be stated that it is likely that a part of the lack of viability may have been due to the eggs coming directly in contact with the oil on the bodies of the ticks.

THE EFFECT OF ARSENIC ON TICKS.

Arsenic acts on ticks entirely as a result of its toxic properties. The avenues by which the arsenic gains entrance to the bodies of ticks have not been determined. As pointed out in Bulletin 144, Bureau of Animal Industry, the possible ways are (1) through the mouth; (2) through the breathing pores; (3) through other openings of the body; or (4) by absorption through the cuticle. Arsenic in entering by way of the mouth may enter in two ways, namely, directly, or indirectly through the blood or lymph. Under other openings of the body may be mentioned the anus, the genital pore in adult ticks, the cephalic gland in the female and nymph, and glandular openings in the cuticle.

It is not likely that any dip enters the body through the anus or genital openings, as these are kept tightly closed. The cephalic gland, located beneath the scutum and having its opening situated in the soft chitin between the scutum and the base of the rostrum, offers a possible point of entrance. This gland secretes a viscous fluid which causes the eggs to adhere together and protects them from loss of moisture by evaporation. Anything that would interfere with the function of this gland would affect materially the viability of the eggs deposited. The openings of the tegumentary glands, which are numerous, furnish another means of entrance of arsenic through the chitinous covering of the body.

The porose areas, two in number, located on the dorsal side of the base of the rostrum, furnish another possible point of entrance in the case of adult females. According to Bonnet[1] the punctations of these areas are pores passing through the chitin, each of which communicates with an exposed nerve cell lying beneath the chitinous covering, and which is the termination of one of the fibers of a nerve adjacent to the area. As these nerve cells are without cell membranes and are unprotected in any way, it would appear that the porose areas constitute a most vulnerable point in the body of the tick for the entrance of arsenic.

[1] Bonnet, Amédée. Recherches sur l'anatomie comparée et le développement des ixodidés. Annales de l'Université de Lyon, n. s., I: sc. méd., fasc. 20. Lyon and Paris.

CATTLE-DIPPING EXPERIMENTS TO ASCERTAIN THE PROTECTIVE ACTION OF ARSENICAL DIPS.

EXPERIMENT NO. 1.—CATTLE EXPOSED TO INFESTATION AT VARIOUS INTERVALS FROM A FEW HOURS TO FOUR WEEKS AFTER DIPPING.

In this experiment the arsenical dip used was made on April 9, 1912. No pine tar was used, for the purpose of avoiding any repellent action that it might contribute to the dip. The dip was made in the usual way, the formula being 8 pounds arsenic trioxid and 24 pounds of sodium carbonate to 500 gallons of dip. Samples of the dip were taken on three dates for analysis. The analyses[1] follow:

Analyses of arsenical dip used in experiment No. 1.

Date.	Actual As_2O_3.	Total As_2O_3.
1912.	*Per cent.*	*Per cent.*
Apr. 29	0.1847	0.1851
May 16	.1841	.1901
June 20	.1229	.2022

It is seen that at the time of the first dipping there was present in the bath 0.1847 per cent of As_2O_3 and a total amount of arsenic equivalent to 0.1851 per cent of arsenic trioxid, and on June 20, two days after the last dipping, 0.1229 per cent of As_2O_3 and a total amount equivalent to 0.2022 per cent arsenic trioxid. The actual amount of As_2O_3 present had decreased during the course of the dipping about one-third through oxidation, and the percentage of total arsenic had increased slightly as a result of evaporation.

Each animal was kept in the dip two minutes.

There were 12 animals (calves) in the experiment, all being northern cattle (nonimmune). Each animal was infested with the seed ticks hatched from 2½ grams of eggs. The seed ticks used were the progeny of engorged females (*Margaropus annulatus*) collected at Fort Worth, Tex., May 3, 1912.

Previous to the time the calves were infested they were kept in a yard provided with shelter, which they might seek when they desired. On the day of infestation (June 18) they were removed to another yard where there was no shelter and kept there until the following noon, when they were placed under cover, where they remained until the close of the experiment. During the night and the forenoon following infestation the animals were out in a light rain.

Lot 1. Calves Nos. 942 and 947.—June 18, 1912, the calves were dipped once in an arsenical dip containing a total amount of arsenic equivalent to 0.2022 per cent As_2O_3 and containing arsenic in the form of As_2O_3 to the extent of 0.1229

[1] All analyses referred to in this bulletin were made by the Biochemic Division of the Bureau of Animal Industry.

per cent. Each calf, as soon as dry, was infested with seed ticks. They were kept under observation until July 22. No ticks developed, and there was no fever.

Lot 2. Calves Nos. 939 and 941.—The calves were dipped four times at intervals of two weeks, on May 7 and 21. and June 4 and 18, in an arsenical dip. They were infested with seed ticks on the date of the last dipping as soon as they were dry. They were under observation until July 22. No ticks developed, and there was no fever.

Lot 4. Calves Nos. 940 and 944.—These were dipped four times at intervals of two weeks (April 30, May 14 and 28, and June 11) in an arsenical dip. On June 18, one week after the last dipping, they were infested with seed ticks. Both animals became infested with ticks and developed Texas fever. Calf 940 died of the fever July 9. This animal became heavily infested with ticks, and at the time of death most of the ticks had molted the second time. A few nymphs were present. The adult ticks ranged from those just molted to some about one-third engorged. Calf 944 became only lightly infested with ticks. Nine hundred and six adult ticks were collected. the last ones being removed on July 30. The animal recovered from the fever.

Lot 5. Calves Nos. 948 and 949.—These were dipped in an arsenical dip four times at intervals of two weeks, on April 23, May 7 and 21, and June 4. Two weeks following the last dipping, that is, on June 18, they were infested with seed ticks. Both contracted Texas fever, and No. 948 died July 11. This calf became moderately infested with ticks. At the time of death most of the ticks were in the adult stage and ranged from the newly molted to the engorged condition. Calf 949 became heavily infested with ticks. The last adults were removed August 1. In all, 4,884 ticks were collected from this animal.

Lot 6. Calves Nos. 943 and 945.—This lot was dipped four times (April 9 and 23, May 7 and 21), at intervals of two weeks, in an arsenical dip. Four weeks after the last dipping (June 18) they were infested with seed ticks. Calf 943 became heavily infested with ticks and calf 945 acquired a moderate infestation. Both contracted Texas fever, and calf 945 died July 8. The stages present were nymphs and young adults. In the case of calf 943 the last tick was collected on August 3, and in all 5,715 ticks were removed.

Lot 3 (control). Calves Nos. 946 and 950.—These animals were not dipped. They were infested with seed ticks on the same date as the other lots (June 18). Both animals became heavily infested with ticks and contracted Texas fever. Calf 946 died July 6. It was heavily infested with nymphs and young adults. Calf 950 was nearly dead July 15 and was killed. Up to the time the animal was killed, 2,960 adult ticks had been collected.

OBSERVATIONS ON ENGORGED TICKS REMOVED FROM THE ANIMALS.

These ticks were kept in Petri dishes.

Ticks removed from calf 944 (lot 4).—July 12, 70 engorged and nearly engorged ticks removed. All but one of the ticks deposited eggs. A good many eggs shriveled. Most of the ticks died either without ovipositing or before oviposition was completed. About 60 per cent of the eggs hatched.

July 15, 13 engorged ticks removed. All but one oviposited. Some eggs shriveled. Thirty-one per cent of the ticks died either without ovipositing or before oviposition was completed. About 10 per cent of the eggs hatched.

July 16, 55 engorged and nearly engorged ticks removed. All oviposited except four. Sixty-four per cent of the ticks died either without ovipositing or before oviposition was completed. About 95 per cent of the eggs hatched.

July 17, 15 engorged ticks removed for study. All oviposited. Seventy-three per cent of the ticks died before oviposition was completed. About 10 per cent of the eggs hatched.

July 18, 20 engorged females removed. All oviposited. Sixty per cent died before oviposition was completed. About 50 per cent of the eggs hatched.

July 19, 25 engorged and nearly engorged ticks removed. All but one oviposited. Small number of eggs deposited. Eggs were scattered and many shriveled. Twenty-four per cent of the ticks died either without ovipositing or before oviposition was completed. Less than 1 per cent of the eggs hatched.

Ticks removed from calf 949 (lot 5).—July 12, removed 336 engorged and nearly engorged ticks. Lot No. 1 of 100 ticks: All but two oviposited. About half of the ticks died either without ovipositing or before oviposition was completed. About 95 per cent of the eggs hatched. Lot No. 2 of 100 ticks: All but one oviposited. About 50 per cent of the ticks died either without ovipositing or before oviposition was completed. About 95 per cent of the eggs hatched. Lot No. 3 of 136 ticks: All but three oviposited. About half of the ticks died either without ovipositing or before oviposition was completed. About 90 per cent of the eggs hatched.

July 15, removed 49 engorged ticks. All oviposited. About 33 per cent of the ticks died before oviposition was completed. About 95 per cent of the eggs hatched.

July 16, removed 119 engorged females. All but six oviposited. About 65 per cent of the ticks died either without ovipositing or before oviposition was completed. About 85 per cent of the eggs hatched.

July 17, removed 30 engorged ticks. All oviposited. About 30 per cent died before oviposition was completed. About 90 per cent of the eggs hatched.

July 18, removed 30 engorged females. All oviposited but one. Forty-three per cent died either without ovipositing or before oviposition was completed. About 95 per cent of the eggs hatched.

July 19, removed 118 engorged and nearly engorged females. All oviposited. About 33 per cent died before oviposition was completed. About 90 per cent of the eggs hatched.

Ticks removed from calf 943 (lot 6).—July 12, removed 200 engorged and nearly engorged ticks. Lot No. 1 of 100 ticks: All but five ticks oviposited. About 65 per cent of the ticks died either without ovipositing or before oviposition was completed. About 95 per cent of the eggs hatched. Lot No. 2 of 100 ticks: All oviposited. About half of the ticks died before oviposition was completed. About 95 per cent of the eggs hatched.

July 15, removed 35 engorged ticks. All oviposited but one. More than half of the ticks died either without ovipositing or before oviposition was completed. About 95 per cent of the eggs hatched.

July 16, removed 116 ticks. All but five oviposited. About half of the ticks died either without ovipositing or before oviposition was completed. About 95 per cent of the eggs hatched.

July 17, removed 20 engorged females. All oviposited. Thirty per cent of the ticks died before oviposition was completed. About 25 per cent of the eggs hatched.

July 18, removed 30 engorged ticks. All but one oviposited. Ten per cent of the ticks died either without ovipositing or before oviposition was completed. About 75 per cent of the eggs hatched.

July 19, removed 49 engorged and nearly engorged females. All but one oviposited. Fourteen per cent of the ticks died either without ovipositing or before oviposition was completed. About 50 per cent of the eggs hatched.

Ticks removed from calf 950 (lot 3, control).—July 12, removed 200 engorged and nearly engorged ticks. Lot No. 1 of 100 ticks: All oviposited. Ninety-seven

per cent of the ticks died before oviposition was completed. About 90 per cent of the eggs hatched. Lot No. 2 of 100 ticks: All but one oviposited. Ninety-five per cent of the ticks died either without ovipositing or before oviposition was completed. About 90 per cent of the eggs hatched.

July 15, removed 26 engorged and nearly engorged ticks. All but one oviposited. Most of the ticks died either without ovipositing or before oviposition was completed. About 90 per cent of the eggs hatched.

DISCUSSION OF RESULTS.

Effect on the animals.—It is noted from the above results that cattle dipped four times at intervals of two weeks in an arsenical dip (lot 2) and subjected to infestation as soon as dry following the last dipping were protected from infestation. It was found that cattle dipped only once (lot 1) and subjected to infestation as soon as dry were likewise protected from infestation. It is therefore seen that arsenic absolutely protected animals from infestation when exposed immediately following a dipping and that one dipping was just as effective in producing this result as a number of dippings at intervals of two weeks. Since none of the animals contracted Texas fever, we are led to conclude that the ticks failed to infect the animals before they were killed by the arsenic.

It was also found that cattle dipped four times at intervals of two weeks and infested one (lot 4), two (lot 5), and four weeks (lot 6) after the last dipping, became infested with ticks and all contracted Texas fever. Of the six animals in these lots, three (50 per cent) died of fever, whereas, as already mentioned, there was no fever or deaths in the lots infested immediately after dipping. In the control lot (lot 3) both animals became infested with ticks and died of Texas fever.

Effect on the ticks.—Observations were made from time to time on the animals for the purpose of noting any ticks that might show evidence of arsenical poisoning. Only a very few dead and abnormal ticks were found. A few dead females were noted on calf 949, a good many abnormal young females, and a few dead ticks on calf 943, and some abnormal young females on calf 945. It is possible that these ticks were affected by arsenic absorbed from the skin of the animal, but their number, compared with the total number of ticks present, was so small that it may be said that the arsenic, if active at all, had a negligible effect on the nymphal and adult stages of the tick. Whether this was also true of the larval stage of the tick can not be said, since it was not practicable to make observations on this stage on account of their small size and the difficulty in finding them.

An interesting abnormality was found in some engorged females taken from two of the dipped animals. Ten of them were taken from calf 949 and one from calf 943. These ticks were plump and trans-

parent. The dark intestinal branches and the Malphigian tubes could be plainly seen lying in the lower portion of the body cavity. On puncturing the body wall a clear fluid issued from the body cavity. Five of the ticks from calf 949 were set aside for special observation. All began ovipositing, but egg-laying progressed very slowly and only a few eggs were deposited. None of the eggs hatched, although some of them developed nearly to the point of hatching.

Nothing final was determined with regard to whether there is any relation between the number of ticks developing on animals and the duration of the interval elapsing between the last dipping and the date of infestation.

For the purpose of obtaining data on this point approximately the same number of ticks were placed on all the cattle—that is, larvæ that hatched from 2½ grams of eggs. In the case of the two animals infested one week after the last dipping (lot 4), one became heavily infested and the other lightly infested; in the case of those infested two weeks after the final dipping (lot 5), one became moderately infested and the other heavily infested; and in the case of the two animals infested four weeks after the last dipping (lot 6), the result was the same, one becoming heavily infested and the other moderately infested. The two control animals became heavily infested with ticks.

An attempt was made to obtain additional and more definite information on the above point by collecting as far as possible all the engorged ticks maturing on the animals. This was interfered with, however, by the death of five of the eight animals that became infested, including the two control animals. The results from the three animals that survived are as follows:

Number of engorged female ticks collected from surviving animals.

No. of animal.	Interval between last dipping and infestation (weeks).	Number of engorged females collected.
944	1	906
949	2	4,884
943	4	5,715

While it would appear from this that the infestation becomes intensified as the interval increases following the last dipping, the data are not adequate for drawing conclusions on this point.

The observations made on the engorged females removed at intervals from the animals (p. 17) were for the purpose of determining whether any arsenic absorbed by the tick might destroy it after it

had dropped from the host, or, failing this, whether it would influence oviposition or affect the viability of the eggs.

The results on mortality obtained from the ticks collected from four animals are as follows:

Lot 4 (calf 944), about 50 per cent died.
Lot 5 (calf 949), about 40 per cent died.
Lot 6 (calf 943), about 50 per cent died.
Lot 3 (calf 950, control), about 95 per cent died.

It is noted that the mortality of the ticks from the undipped control animal is higher than for the dipped animals. The mortality in all cases is abnormally high and is very likely due to the fact that all of the animals suffered from Texas fever, and this, either because of the well-known changes in the blood or some other cause, such as, for example, an excessive invasion with *Piroplasma bigeminum*, had a deleterious effect on the ticks. The results, therefore, do not make it possible to draw any conclusions with regard to what part, if any, arsenic played in the mortality.

After obtaining the above results the writer made some observations on engorged females collected from undipped animals suffering from Texas fever in an experiment conducted by Drs. Schroeder and Cotton, of the Bureau of Animal Industry. The data obtained from these ticks are as follows:

Mortality of engorged ticks collected from animals suffering from Texas fever.

No. of animal.	Number of ticks removed.	Mortality.
		Per cent.
808	1,277	10
792	665	12
684	978	7
803	579	17
742	372	32

The mortality here is not nearly as high as in the above instances. Unfortunately, no ticks from animals not suffering from fever were available for controls, but it is known as the result of a wide experience with ticks from normal animals that the mortality is abnormally high.

The percentage of the engorged females that oviposited is given in the following table. The ticks were collected from each animal from July 12 to 19, inclusive, and kept under observation.

Percentage of engorged ticks ovipositing.

Number of animal from which collected.	Number of ticks.	Ticks ovipositing.
		Per cent.
944 (lot 4)	198	96
949 (lot 5)	682	98
943 (lot 6)	450	97
950 (lot 3, control)	226	99

It is noted from the above table that the ticks ovipositing from the dipped animals range from 1 to 3 per cent lower than those from the undipped animal (control). This difference, however, is so slight as to have no significance, and it may be concluded that any arsenic absorbed by the ticks failed to be sufficiently active to prevent oviposition.

The percentage of eggs hatched in the case of the various lots of ticks collected from animals that survived long enough for ticks to mature is as follows:

Percentage of eggs hatching.

Date collected.	Calf 944 (lot 4).	Calf 949 (lot 5).	Calf 943 (lot 6).	Calf 950 (lot 3, control).
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
July 12	60	95, 95, and 90	95 and 95	90 and 90
July 15	10	95	95	90
July 16	95	85	95	....
July 17	10	90	25	....
July 18	50	95	75	....
July 19	1	90	50	....

The percentage in the three dipped animals (944, 949, and 943) ranged from 1 to 95, 85 to 95, and 25 to 95, respectively, as compared with 90 per cent in the three lots from the control animal.

Because of the fact that it was necessary to kill the only remaining control animal (calf 950) on July 15, there were no undipped ticks available for comparison with those collected from the dipped animals on July 16 to 19, inclusive.

By comparing the percentages for the dipped animals with those of the control for July 12 and 15, it is noted that the percentage of eggs hatching from ticks from calf 944, infested one week after dipping, is low, while the percentage for each of the other two animals, infested two and four weeks after dipping, is normal. It is noted that in general the percentages for the remaining dates range lower for calf 944 than for the other animals. Unfortunately the percentages for the dipped animals can not be averaged for purposes of comparison, because the number of eggs in the different lots varied greatly.

It is evident, however, that while arsenic may have been responsible for a decrease in the viability of the eggs in the case of calf 944, there has been no such effect in the case of the ticks from the other two dipped animals, and, on the whole, it seems safe to conclude that arsenic has played no important part in determining the percentage of eggs that hatched.

It should be borne in mind that this result is not contrary to those recorded in Bulletin 144 of the Bureau of Animal Industry, where ticks subjected to the direct influence of arsenic were found to lay eggs

that, as a rule, did not hatch. The ticks in the present tests were subjected only to the indirect action of arsenic, as they were not applied as seed ticks until one to four weeks after the animals were dipped.

EXPERIMENT NO. 2.—CATTLE EXPOSED TO INFESTATION TWO AND ONE-HALF HOURS AND TWO DAYS AFTER DIPPING.

After having shown by experiment No. 1 that arsenic has a protective action which lasts for less than a week, it was determined to conduct another experiment for the purpose of determining the length of time that arsenic will protect. It was also thought necessary to repeat the tests showing that arsenic protected against immediate infestation, since there was a possibility that the results obtained might have been accidental. One thing that threw doubt on the last-mentioned result was the fact that the animals, following the last dipping and the infestation (carried out on the same day), were out in a slow rain, which it was thought might have kept the arsenic in solution on the skin and created a condition much as if the ticks had been subjected for some hours to an arsenical dip. In order to obviate this possibility in the following experiment, the dipped animals, as soon as dry, were placed at once under shelter and kept there until the close of the experiment.

In experiment No. 1 the animals were kept submerged in the dip for two minutes. As this is longer than an animal remains in the dip when passing through the ordinary dipping vat, it was determined in the following experiment, in addition to dipping animals for 2 minutes, to dip others for 20 seconds, which comes nearer to the time required to pass through a vat.

The dip used in experiment No. 2 was made on July 29. It was made according to the usual formula of 8 pounds of arsenic to the 500 gallons. An analysis of a sample taken on the date the dip was made showed it to contain 0.1652 per cent arsenic trioxid. As in experiment No. 1, no pine tar was used. Twelve calves were used in the experiment, divided into five lots as described below. All of the cattle were dipped on August 1. Lots 1, 2, and 5 were infested with the progeny of female ticks collected at Fort Worth, Tex., June 24, 1912. Lots 3 and 4 were infested with the progeny of females received from Fort Worth, June 29, 1912.

Lot 1. Calves Nos. 957, 947, and 958.—Dipped two minutes in an arsenical dip. Infested with seed ticks two and one-half hours after dipping. No ticks developed. Calves 957 and 947 developed no fever. Calf 958 showed a temperature and in all probability had an attack of Texas fever. Calf 947 following dipping suffered from anorexia and bloat. On August 26 the animal developed a gastric fistula located on the underside of the abdomen. Food and drops of liquid were issuing from it. On August 29 the animal was killed. The fistula was found to extend into the fourth stomach. The mucous folds in the region of the fistula were hypertrophied, hemorrhagic, and sloughing away. This was clearly a case of arsenical poisoning.

Lot 2. Calves Nos. 927, 939, and 928.—Dipped 20 seconds in an arsenical dip. Infested with seed ticks two and one-half hours after dipping. No ticks developed on these animals. There was no fever. Calf 939 died August 20. On post-mortem there was an enteritis, principally of small intestine and cecum. There was congestion of lungs and a slight congestion of kidneys. Smears from spleen and liver contained no piroplasma.

Lot 3. Calves Nos. 955 and 942.—Dipped two minutes in an arsenical dip. Infested two days after dipping (August 3) with seed ticks. No ticks developed on these animals. Calf 942 showed a rise in temperature from the ninth to the twelfth day after infestation. On August 6 calf 955 died of arsenical poisoning. The liver was yellow (fatty degeneration) and very friable. There were large hemorrhages in the endocardium of the right side, also large hemorrhagic areas in all four stomachs. An enteritis was present. There were edematous areas in wall of stomach and intestine. The capsule of the spleen was much injected. All blood vessels were injected and the blood was very dark. There were no lesions in the mouth.

Found a number of larvæ on the skin, all of which were dead. Two of these were observed with certainty to be attached.

Lot 4. Calves Nos. 956 and 941.—Dipped 20 seconds in an arsenical dip. Infested with seed ticks two days after dipping (August 3).

August 16 found a very young nymph on calf 941.

August 20 removed above tick for examination, and determined that it was a specimen of *Margaropus annulatus*. No other ticks were found. No ticks developed on calf 956. This animal showed a temperature from the sixth to the twelfth day. Calf 941 showed no temperature.

Lot 5 (control). Calves Nos. 926 and 938.—Undipped. August 1 infested with seed ticks. Both of these animals became heavily infested with ticks and showed a rise in temperature. Calf 926 was down August 22. Had been down since the previous day. The animal was greatly emaciated. It was killed. Heavily infested with ticks. There were no lesions of Texas fever. Smears from liver and spleen contained no piroplasma. On August 16 both of the above animals were treated with a subcutaneous injection of 150 c. c. of a 1 per cent solution of trypan blue. Calf 938 was killed August 30 for the purpose of noting whether the stain from the trypan blue still persisted. On post-mortem both carcasses were found to be extensively stained. The parts stained were as follows: The skin; subcutaneous and intermuscular connective tissue; parietal and visceral pleura and the peritoneum; epicardium, endocardium, and pericardium; trachea; bronchi and smaller air tubes; cartilage of joints; capsule and trabeculæ of spleen; cortex of kidneys, and the veins and arteries. A pale-bluish fluid was noted in some of the mesenteric lymph glands.

The post-mortems were made 6 and 14 days after the trypan-blue solution was injected.

DISCUSSION OF RESULTS.

It is noted from the above results that animals when dipped once in an arsenical dip for two minutes (lot 1) and for 20 seconds (lot 2) were protected from infestation when exposed two and one-half hours after dipping. This occurred when the animals were not exposed to rain and the arsenic on the skin was not kept in a more or less dissolved state for some time, as was probably the case in experiment No. 1.

It is also seen (lots 3 and 4) that animals dipped for two minutes and for 20 seconds were protected from infestation when exposed two

full days after dipping. It is believed that the one young nymph found 13 days after infestation on calf 941 could not have been a larva that attached itself at the time of infestation, since its stage of development was considerably behind that of a tick nearly two weeks old. It is likely that this tick failed to attach itself, became dislodged from one of the calves after they were placed in the experimental paddock, and some days later succeeded in attaching itself. It is possible, however, that this tick attached itself on the day of infestation. The writer obtained (Bul. 130, Bureau of Animal Industry) in experiments conducted in 1908 a maximum period of 14 days for the larval stage, although it is possible that here also the tick failed to become attached at once. The usual larval period is from five to nine days.

It is noted that both of the control animals became heavily infested with ticks.

DEATH OF ANIMALS FROM ARSENICAL POISONING.

In the above experiment two animals (calves 939 and 955) died from arsenical poisoning, and it was necessary to kill another (calf 947) suffering from a gastric fistula due to arsenical poisoning. A number of the other dipped animals suffered for a time with anorexia, and some had temperatures that were puzzling and could not with certainty be attributed to Texas fever. It is possible that they also suffered from arsenical poisoning. One of the undipped (control) animals became sick, and it was necessary to kill it. The cause of its sickness was not clear, but was very probably Texas fever. Although it showed a temperature, there were no lesions of Texas fever on post-mortem, and the piroplasma could not be demonstrated on microscopic examination. However, six days previously it had been injected with a solution of trypan blue. This treatment may have eliminated the organism, which, if present at all, was probably present in small numbers, as the animal was a calf.

The death of three animals from arsenical poisoning and the sickness of a number of others, due possibly to the same cause, was puzzling to the writer for a time, especially in view of the fact that this bureau had conducted experimental dippings in arsenic for a number of years at the experiment station without a single loss. The analysis of the dip used showed an arsenic content of 0.1652 per cent. This was much too low for the quantity of arsenic used. The percentage of arsenic should have been 0.1925. It is evident that all of the arsenic was not dissolved when the dip was made. The most probable explanation of the poisoning is that there was undissolved arsenic in the dip, and the animals while being dipped swallowed enough to cause poisoning. Support is lent to this view by the fact that much difficulty was experienced in dissolving the

arsenic, because the water was not kept boiling. After all the arsenic had apparently gone into solution and the strong solution was added to the water in the vat, clumps of white particles were seen floating in the water. The writer at the time assumed these to be masses of calcium carbonate thrown down by the sodium carbonate. It was shown that this could not have been the case, however, by observations made when the batch of dip was prepared for the next experiment (No. 3). Water from the same source was used, and no such precipitate was thrown down by the sodium carbonate. The only conclusion that remains, and this is supported by the analysis referred to above, is that the particles observed were undissolved arsenic. If the conclusions here drawn with regard to the cause of the poisoning are correct, the extreme danger of having undissolved arsenic in a dip is indicated, and it is possible that an explanation of losses in certain instances has been furnished.

STAINING OF TISSUES OF ANIMALS TREATED WITH TRYPAN BLUE.

The extensive and intense staining of the tissues of animals treated with subcutaneous injections of a 1 per cent solution of trypan blue has been previously noted in the post-mortems of animals Nos. 926 and 938. The post-mortems were made 6 and 14 days after the treatment. It would be of some practical interest to determine how long the staining will persist in treated animals, since it appears that it might interfere with the use of animals for beef purposes for a considerable period.

EXPERIMENT NO. 3.—CATTLE EXPOSED TO INFESTATION FIVE DAYS AFTER DIPPING.

Following experiment No. 2 another was undertaken to determine whether arsenic will protect animals from infestation five days after dipping.

The dip used in experiment No. 3 was made up in the proportions of 8 pounds of arsenic trioxid and 24 pounds of sodium carbonate to 500 gallons of water. No pine tar was used. The dip was made August 29, and an analysis showed it to contain 0.1869 per cent of arsenic trioxid. The results of the experiment were as follows:

Lot 1. Cattle Nos. 830 and 860.—Dipped August 29, 20 seconds. September 4 (five days later) infested cattle with progeny of ticks collected August 8, 1912, at Fort Worth, Tex. Both animals became grossly infested with ticks and contracted Texas fever. On September 20 animal 830 died. Post-mortem showed typical lesions of Texas fever.

Lot 2 (control). Cattle Nos. 855 and 858.—Undipped. September 4 infested with progeny of ticks collected August 8 at Fort Worth, Tex. Both animals became grossly infested with ticks and contracted Texas fever.

DISCUSSION OF RESULTS.

It is shown by the above experiment that animals dipped in an arsenical dip containing 0.1869 per cent of arsenic trioxid are not protected from infestation when exposed five days after dipping. It also appears that the degree of infestation was not influenced in any way by the dipping in arsenic.

THE METHOD BY WHICH ANIMALS ARE PROTECTED AGAINST TICK INFESTATION.

There are two ways in which it is conceivable that arsenic protects against infestation: that is, by rendering the skin unpleasant to the tick, and by poisoning the tick after it attaches. Support is lent to the latter view by the fact that dead larvæ were found attached to the skin of calf 955 at the time of its death three days after it was infested. It was not practicable to multiply observations similar to the above because of the great length of time consumed in locating the seed ticks on the animals.

The results of the experiments, however, have proved conclusively that the protection is due to the larvæ being killed and not to their being repelled. The animals when seed ticks were applied to them were placed in a special pen set aside for that purpose, and several hours later, after the ticks had had time to attach themselves, were placed in the tick-free pens they were to occupy during the experiment. If arsenic possessed simply a repellent action effective for two days, complete protection would not have resulted as occurred in the experiments, since the larvæ would either have wandered about over the hair of the animals or have become dislodged, only to become attached later when the repellent action of the dip had ceased to be effective. It is therefore seen that if we were dealing with a repellent action instead of a toxic action at least a certain degree of infestation must have resulted in the case of all of the dipped animals, unless, of course, all the seed ticks left the animals between the time the ticks were applied and the animals were placed in their permanent pens, a possibility that is too remote to be worthy of consideration.

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